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Previews

SMIP-016 in Action: CD37 as a Death Receptor

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CD37 is a tetraspannin that triggers cell death and is a potential therapeutic target in cancers. In this issue of *Cancer Cell*, Lapalombella et al. show that CD37 is tyrosine phosphorylated following engagement by a bivalent engineered antibody fragment that binds CD37 and activates both SHP-1-dependent apoptotic signaling and PI3K-AKT-mediated survival signaling.

Small modular immuno-pharmaceuticals (SMIP) are disulfide-linked dimers of single-chain proteins comprised of one antigen binding VH/VL, a connecting hinge region, and an Fc (fragment, crystallizable) region (CH2-CH3) (Figure 1). Because of their smaller size, these candidate therapetics may have better tissue penetration than monoclonal antibodies (mAbs). SMIP-016 is a dimeric recombinant single-chain polypeptide engineered to exhibit the full binding activity of an anti-CD37 mAb but at one-third less of the size (Zhao et al., 2007). Previous studies have shown that SMIP-016 is a potent inducer of apoptosis and antibody-dependent cellular cytotoxicity in B-cell leukemia/lymphoma cell lines and primary chronic lymphocytic leukemia (CLL) cells and is superior to the therapeutic antibodies used in these diseases (Zhao et al., 2007). TRU-016 is a humanized variant of SMIP-016 and is currently undergoing clinical trials for patients with CLL (http://www.clinicaltrials.gov).

CLL is the most common type of leukemia. To date, the anti-CD20 mAb Rituximab remains the most widely used mAb in the treatment of CLL. A high response rates of 51% with 4% complete response (CR) were achieved when Rituximab was used in previously untreated patients with CLL (Hainsworth et al., 2003). SMIP-016 acts by a mechanism distinct from Rituximab, inducing caspase independent and tyrosine phosphorylation-dependent apoptosis (Zhao et al., 2007). Thus, it was not surprising that TRU-016 and Rituximab showed complementary activity (Robak et al., 2009).

CD37 is a four transmembrane glycoprotein expressed at high levels on B cells and to a lesser extent on T cells and myeloid cells (Barrena et al., 2005). The physiological ligand for CD37 is unknown, though it is shown that CD37 interacts with the C-type lectin dectin-1. Dectin-1 recognizes β-glucans found in cell walls of fungi. It is possible that together with dectin-1, CD37 forms a pattern-recognition-receptor for pathogen-associated molecule pattern. Indeed, $CD37^{-/-}$ mice are better protected from *Candida albicans* infection than WT mice (van Spriel et al., 2009).

CD37 belongs to the tetraspanin protein family and associates with other tetraspanins, such as CD53, CD81, and CD82 to form multiprotein complexes, the so-called tetraspanin web, on cell surfaces (Tarrant et al., 2003). Like other tetraspanins, CD37 has short cytoplasmic tails (8–14 aa) that lack typical signaling domains. $CD37^{-/-}$ mice display impaired T cell-dependent antibody responses (Knobeloch et al., 2000) and increased dendritic cell antigen-presenting capacity (Sheng et al., 2009).

In this issue of *Cancer Cell*, Lapalombella et al. (2012) show that CD37 has intrinsic tyrosine-based signaling capacity that is important for SMIP-016 induced apoptosis in primary CLL cells. SMIP-016-induced apoptosis in CLL patients' samples depends upon tyrosine phosphorylation (Zhao et al., 2007). The authors started their investigation by identifying tyrosine phosphorylated proteins associated with CD37 upon SMIP-016 stimulation. One of the targets they identified is the SH-2-containing tyrosine phosphatase SHP-1. They showed that reducing SHP-1 expression dramatically decreases SMIP-016 induced apoptosis in CLL samples. SHP-1 downregulates signaling pathways that promote proliferation, and it is considered a tumor suppressor (Wu et al., 2003). The authors then explored how SHP-1 is recruited to CD37. They noticed that the N-terminal cytoplasmic tail of CD37 contains a weak S/I/V/LxYxxI/V/L immuno-tyrosine inhibitory motif (ITIM) that is known to bind the SH2 domains of SHP-1. Using biochemistry, mass spectrometry, and mutagenesis approaches, the authors convincingly demonstrate that CD37 itself is tyrosine phosphorylated upon SMIP-016 stimulation. Furthermore, most of this phosphorylation occurs on the N-terminal ITIM motif. A Y^{13} to F^{13} CD37 ITIM mutant does not bind SHP-1, and SMIP-016 is less effective in killing cells expressing this mutant.

Exploring other functional consorts, the authors found that the C-terminal cytoplasmic tail of

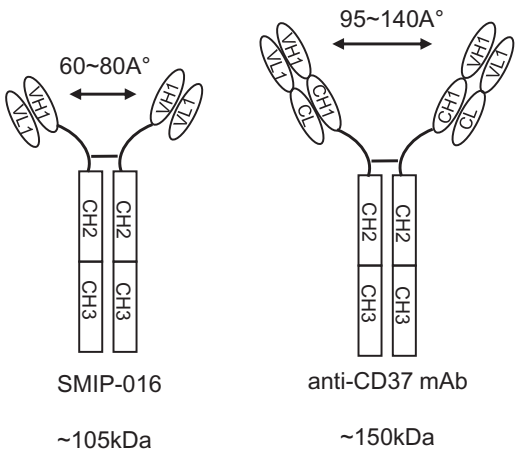

Figure 1. SMIP-016 Is an Anti-CD37 Polypeptide
Comparison of SMIP-016 and an anti-CD37 mAb. VH, variable heavy chain; VL, variable light chain; CH, constant heavy chain.

CD37, which contains two additional tyrosines Y^{274} and Y^{280}, somehow inhibits CD37 phosphorylation and the cytotoxic effects of SMIP-016. This led to the finding that SMIP-016 also activates the PI3K-AKT proliferative signaling pathway. Treatment of cells with the PI3K inhibitor LY294002 or deleting the C-terminal tail of CD37 increases SMIP-016-induced killing. In summary, SMIP-016 simultaneously activates both SHP-1 mediated death signaling and PI3K-AKT mediated survival signaling.

The study of Lapalombella et al. (2012) not only provides deeper insight into the molecular mechanisms of SMIP-016 action but may also help guide current and future clinical trials using TRU-016. For example, the current study reveals an opposing role for PI3K and an absolute requirement for SHP-1 expression for efficacy of SMIP-016. Consistent with its tumor suppressor role, expression of SHP-1 is diminished or absent in many leukemias and lymphomas (Wu et al., 2003). Thus, it can be expected that cancers with low or no SHP-1 expression may not respond to TRU-016 treatment. The results from current TRU-016 clinical trials on CLL are expected in the first half of 2013, and in interpreting the outcome, it may be useful to stratify subjects based on the SHP-1 expression level and the PI3K pathway activity in their tumors.

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Opening a New GATAway for Treating KRAS-Driven Lung Tumors

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In a recent issue of *Cell*, Kumar and colleagues uncovered a synthetic lethal interaction between oncogenic KRAS and the transcription factor GATA2 in non-small cell lung carcinoma. Pharmacological inhibition of GATA2-mediated pathways with bortezomib and fasudil results in dramatic tumor inhibition. These observations unveil new armamentaria to fight this deadly disease.

Non-small cell lung carcinoma (NSCLC) has one of the highest incidence and lowest survival rates, a combination that makes this tumor type one of the deadliest human cancers. At least a quarter of NSCLC express a mutant *KRAS* allele that encodes a constitutively active small G protein known to signal through a series of kinases. While these kinases are in principle amenable to pharmacological intervention, a selective treatment for *KRAS* mutant NSCLC is not yet available in the clinic.

During the last decade, there have been significant efforts directed to reproduce the natural history of NSCLC in genetically engineered mouse (GEM) models (Heyer et al., 2010). Recently, these models have been utilized to evaluate potential therapeutic targets. Some of the validated targets include well-known downstream elements of KRAS signaling such as components of the mitogenic RAF/MEK/ERK cascade and the PI3K/AKT survival pathway, most of which are druggable kinases (Gupta et al., 2007; Engelman et al., 2008; Blasco et al., 2011) (Figure 1A). However, these studies cannot be directly extrapolated to the clinic because target ablation occurred during tumor initiation rather than during tumor progression. Moreover, these *Kras* oncogene-driven GEM models retained the full component of tumor suppressors and, hence, do not develop metastatic tumors.

Other studies have attempted to validate pathways less directly linked to KRAS signaling. In one study, elimination of CDK4, but not the other interphase CDKs, elicited a rapid senescence response that resulted in partial tumor regression, an observation validated with clinically available CDK4 inhibitors (Puyol